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Research Article

Antioxidant Activity of Ethanol Extract on Qust Al Hindi (*Saussurea lappa*) Roots Using the FRAP Method

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ABSTRACT: Qust al hindi (*Saussurea lappa*) is a plant that has antioxidant activity, especially in the roots. Qust al hindi contains several chemical compounds including flavonoids, steroids, terpenes, alkaloids, sesquiterpenes, costunolide, dehydrococtus lactones, cynaropicrin, and chlorogenic acid. This study aims to determine the antioxidant activity of the ethanol extract of qust al hindi root using the FRAP method. Samples that had become powder were extracted using the soxhletation method with 96% ethanol and obtained a % rendition of 5.302%. The extract obtained was measured using a UV-Vis spectrophotometer at a maximum wavelength of 718 nm after being mixed with several FRAP reagents, using a quercetin standard. The results of the antioxidant analysis showed that the average antioxidant activity of the ethanol extract of the qust al hindi root (*Saussurea lappa*) using the Frap method was 10.3671 mgQE/g extract. Which means that the antioxidant activity of each g of extract is equivalent to the antioxidant activity of 10.3671 mg of quercetin.

KEYWORDS: Antioxidants; flavonoids; FRAP; Qust al hindi.

1. INTRODUCTION

Qust al hindi *(Saussurea lappa) is an important plant that is widely used in traditional to modern medicine. This plant originates from India.* Qust al hindi is a medicinal plant that is known from several traditional medicinal systems in Persia and India and is known mostly in prophetic medicine. Qust al hindi also acts as a treatment for Covid-19 as it can be used to treat fever, nausea, cough and bronchial asthma (Sukmawati *et al*., 2022)

Qust al hindi is an upright, tall, perennial growing to a height of 1–2 m with erect stems. Sturdy root about 60 cm has a strong and distinctive smell. The cross section clearly shows the periderm where the phloem and xylem are clearly visible. The stems are also sturdy and fibrous. The leaves are stalked and about 1 m long. The flowers are dark bluish purple to black, arranged in the leaf axils. The flower heads are stemless, hard and round in shape, about 3–5 cm in diameter. Fruit about 3 mm long, curved, cupped and compact (Madhuri *et al*., 2012)

In the Kashmir region of northern India, a hot water extract from the root of qust al hindi has been used traditionally for the treatment of asthma, inflammation and rheumatism. Many authors have reported that the root of the Qust al Hindi plant has active molecules with cortisol lowering, bronchodilator, antiulcer, anticancer, anti-inflammatory, antiviral and hepatoprotective effects (Mohamed Saleem *et al*., 2013). Qust al hindi also has several other uses, namely as a perfume and pesticide. In China, the roots are used as a base for incense by making sticks and then burning in temples for worship, and the smoke also functions as a repellent for mosquitoes, gnats, and other flying insects. In India, it is widely used as a traditional medicine for stomachaches, headaches, coughs, colds, throat infections and fever, by drinking a decoction of the roots of this plant (Zahara *et al*., 2014). Qust al hindi root has been extensively suggested for the treatment of inflammation-related ailments such as asthma, chronic gastritis, rheumatoid arthritis, and bronchitis.

Qust al hindihas a variety of active compounds, including flavonoids, steroids, terpenes, alkaloids, sesquiterpenes, costunolide, dehydrococtus lactones, cynaropicrin, and chlorogenic acid (Abd Eldaim *et al*., 2019). The antioxidant activity of *Saussurea lappa* is due to the presence of chlorogenic acid. Chlorogenic acid prevents oxidation and removes free radicals (Singh and Chahal, 2017). The roots contain liquid resin, alkaloids, solid resin, valeric acid salts, astringets and ash. The active constituents of the root are: Essential oil of strong aromatic penetrating and sweet smelling (1.5%); aglucoside and the alkaloids saussurine (0.05%). Kuth root contains resinoids (6%), alkaloids (0.05%), inulin (18%), saussurea lactones (20-25%), fixed oils and minor constituents such as tannins and sugars (Singh and Chahal, 2018)

Antioxidants are defined as inhibitors that inhibit oxidation by reacting with reactive free radicals to form stable, unreactive free radicals (Mu'nisa *et al*., 2013; Fawwaz *et al*., 2023). Antioxidants are substances that can delay, slow down and prevent the oxidation process or neutralize free radicals (Sembiring *et al*., 2016). Qust al hindi is a medicinal plant that is rich in antioxidants. Qust al hindi has a remarkable protective effect through its antioxidant activity. Antioxidants based on how they work are divided into two, namely primary and secondary antioxidants. Primary antioxidants are also called chain-breaking antioxidants, and secondary antioxidants work by inactivating metals, scavenge singlet oxygen and stabilize free radicals (Andarina and Djauhari, 2017). Currently, the need for natural antioxidants is in great demand because synthetic antioxidants have more side effects, such as allergies, asthma, inflammation, headaches, decreased consciousness, and disorders of the eyes and stomach (Maulida *et al*., 2022).

A free radical is an atom or molecule that has one or more unpaired electrons in its outermost orbital. Free radicals can also be found in the environment, some metals such as iron and copper, cigarette smoke, drugs, packaged foods,

additives and others (Syarif *et al*., 2015). Free radicals will react with surrounding molecules to obtain electron pairs to achieve molecular stability. The reaction takes place continuously in the body and if it is not stopped it will cause diseases such as cancer, cataracts, premature aging, heart disease and other degenerative diseases. Compounds needed to neutralize and also prevent damage caused by free radicals are antioxidants. Antioxidants can complement the lack of electrons needed by free radicals and inhibit the chain reaction from the formation of free radicals (Ibrahim *et al*., 2016).

Compounds that have reducing power may act as antioxidants because they can stabilize radicals by donating electrons or hydrogen atoms so that the radical compounds become more stable (Mamonto *et al*., 2014). The Ferric Reducing Antioxidant Power (FRAP) method is a method used to test antioxidants in plants. The advantage of the Ferric Reducing Antioxidant Power (FRAP) method is that it can determine the total antioxidant content of a material based on the ability of antioxidant compounds to reduce Fe^{3+} to Fe^{2+} so that the antioxidant power of a compound is analogous to the reducing ability of that compound.(Maryam *et al*., 2016). The reducing ability of the extract was measured from the ability of the extract to act as an electron donor in the reduction reaction of ferricyanide $[Fe(CN)_6]^{3-}$ to ferrocyanide $[Fe(CN)_6]^{4-}$). By adding Fe^{3+} ions from $FeCl_3$ to ferrocyanide ions from the reduction, a complex compound $(Fe^{3+})_4$ $[Fe^{2+}(CN^-)_6]^3$ will be formed (Murningsih, 2012). The Ferric Reducing Antioxidant Power (FRAP) method is a method for determining antioxidant content by spectrophotometry. UV-Vis spectrophotometry can be used to inform both qualitative and quantitative analysis. Qualitative analysis can be used to identify the quality of drugs or their metabolites. The data generated by UV-Vis Spectrophotometry are in the form of maximum wavelength, intensity, pH and solvent effect, while in quantitative analysis, a radiation beam is applied to the sample (sample solution) and the intensity of the transmitted radiation is measured (Princess, 2015). In general, the spectrophotometer component consists of a radiation source, a monochromator. sample holder and detector connected to a printer or computer (Tukadi, 2016).

The aim of this study was to test the antioxidant activity of the ethanol extract of the roots of the qust al hindi plant using the Ferric Reducing Antioxidant Power (FRAP) method.

2. EXPERIMENTAL SECTION

2.1. Sample collection and processing

This research was conducted experimentally in the Pharmaceutical Chemistry Laboratory of the Indonesian Muslim University. The research sample used was the root of the Qust al Hindi (*Saussurea lappa*) plant. The study was conducted by testing the antioxidant activity of the roots of the Qust al Hindi (*Saussurea lappa*) plant using the FRAP method. Qust al hindi (*Saussurea lappa*) roots are collected and cleaned of dirt and then dried. The dried roots were chopped and then blended into powder, then sieved to obtain a finer sample powder, then stored in a tightly closed container. Qust al hindi root powder was weighed as much as 50 g and then put into a cellulose thimble, then put 300 mL of 96% ethanol into a Soxhlet flask. Soxhletation was carried out at 70°C for 3 hours. The extract obtained was filtered using Whatman filter paper and then concentrated using a rotary vacuum evaporator at 60°C for 30 minutes until the solvent was separated. Then it was thickened over a water bath at 70°C.

2.2. Materials and tools

The materials used in this study were aluminum foil, distilled water, trichloroacetic acid, phosphate buffer pH 6.6, ethanol extract of the roots of the Qust al Hindi (*Saussurea lappa*) plant, 96% ethanol, $FeCl_3$, potassium ferricyanide, filter paper and quercetin. The tools used are glassware (Pyrex), sieve, blender, spray bottle, micropipette, oven, water bath, rotary vacuum evaporator, horn spoon, centrifuge, soxhletation device, UV-Vis spectrophotometer, analytical balance, vortex.

2.3. Reagents

a. Phosphate Buffer Solution 0.02 M (pH 6.6)
   The solution was prepared by mixing 50 mL of 0.2 M potassium dihydrogenphosphate with 16.4 mL of 0.2 N sodium hydroxide then diluted with CO2-free water to exactly 200 mL.
b. 0.1% FeCl3 solution
   The solution was prepared by weighing 0.025 g of FeCl3 and then dissolved with distilled water, then made up to the mark mark on a 25 mL volumetric flask.
c. TCA solution (trichloroacetic acid) 10%
   Weigh 2.5 g of TCA and then dissolve it in distilled water, then make it up to the mark mark in a 25 mL volumetric flask.
d. $K_3Fe(CN)_6$ (potassium ferricyanide) 1 % solution
   As much as 0.25 g of K3Fe(CN)6 then dissolved with distilled water, made up to the mark mark in a 25 mL volumetric flask
e. Blank solution
   1 mL of 96% ethanol solvent was pipetted and then 1 mL of phosphate buffer pH 6.6 and 1 mL of 1% potassium ferricyanide [$K_3Fe(CN)_6$] were added. The mixture was vortexed for 5 minutes, then incubated at 50°C for 20 minutes, then 1 mL of 10% TCA was added. Then it was centrifuged at 3000 rpm for 10 minutes, the top layer

of 1 mL solution was mixed with 1 mL distilled water and 0.5 mL 0.1% $FeCl_3$, after which it was incubated at room temperature for 5 minutes.

2.4. Antioxidant Activity Testing

a. Maximum Wavelength Determination (ג max)

The maximum wavelength was obtained by measuring the absorbance of standard quercetin with a concentration of (35 ppm). 1 ml of the solution was taken, then 1 ml of phosphate buffer pH 6.6 and 1 ml of 1% potassium ferricyanide [$K_3Fe(CN)_6$] were added. The mixture was vortexed for 5 minutes, then incubated at 50°C for 20 minutes, then 1 mL of 10% TCA was added. Then centrifuged at 3000 rpm for 10 minutes, the top layer of the solution was 1 mL mixed with 1 mL distilled water and 0.5 ml $FeCl_3$ 0.1%, then incubated at room temperature for 5 minutes. Then read at wavelengths in the range of 500-800 nm using UV-Vis Spectophotometry.

b. Quercetin Standard Curve Determination

For the preparation of a standard solution, from a stock solution of 1000 ppm, it is diluted to 100 ppm and then a working standard is made with a concentration of 15; 20; 25; 30; 35; and 40 ppm. Pipette 1 mL of each working standard concentration and then add 1 mL of phosphate buffer pH 6.6 and 1 mL of 1% potassium ferricyanide [$K_3Fe(CN)_6$]. The mixture was vortexed for 5 minutes, then incubated at 50°C for 20 minutes, then 1 mL of 10% TCA was added. Then it was centrifuged at 3000 rpm for 10 minutes, the top layer of the solution was 1 mL mixed with 1 mL distilled water and 0.5 ml $FeCl_3$ 0.1%, after which it was incubated at room temperature for 5 minutes. Absorbance was measured at a wavelength of 718 nm on UV-Vis Spectophotometry.

c. Antioxidant Determination of Qust Al Hindi Root Ethanol Extract

As much as 25 mg of the ethanol extract of the root of Qust al Hindi (*Saussurea lappa*) was dissolved in 5 mL of 96% ethanol then pipetted 2 mL and added to 5 mL of 96% ethanol. Then pipetted 1 mL of the solution, added 1 mL of 0.02 M phosphate buffer (pH 6.6) and 1 mL of 1% $K_3Fe(CN)_6$ after that, incubated for 20 minutes at 50°C. After incubation, 1 mL of TCA was added and then centrifuged at 3000 rpm for 10 minutes. Then pipette 1 mL of the top layer into a test tube and add 1 mL of distilled water and 0.5 mL of 0.1% $FeCl_3$. Then incubated at room temperature for 5 minutes. The absorbance of the solution was measured at a wavelength of 718 nm on a UV-Vis spectrophotometer. Replicated three times with the same treatment.

3. RESULTS AND DISCUSSION

Antioxidants are substances that can delay, slow down and prevent the oxidation process or neutralize free radicals (Sembiring et al., 2016). The Ferric Reducing Antioxidant Power (FRAP) method can reduce Fe^{3+} ions to Fe^{2+} in free radicals by donating their free electron pairs. In this study, the compound used as a comparison was quercetin, because it functions as a secondary antioxidant by capturing free radicals and preventing chain reactions from occurring.

In this study, the extraction method used was soxhletation, because it is one of the recommended extraction methods for hard-textured samples such as qust al hindi roots. The principle of the soxhlet extraction method is that when the extractor liquid is heated it can evaporate then the extractor liquid vapor rises through the side pipe of the condenser (reverse cooling), the presence of the condenser can condense the vapor so that the steam will fall back through the thimble filled with powder so that the thimble will be filled with the solvent slowly -land. The filter liquid will wet the powder in the thimble then the filter will dissolve the active substance contained in the powder, when the liquid reaches the surface of the siphon, the liquid will fall back into the round bottom flask. The filter liquid can carry the extracted compounds (Agus *et al*., 2023). The solvent used is 96% ethanol. Ethanol has the same properties as methanol, but it is not as toxic as methanol (Ramdja *et a*l., 2009)·Ethanol is the maximum solvent in attracting phenolic compounds when compared to water or a mixture of ethanol and water. Soxhletation extraction to obtain cycle droplets that are colorless or perfectly filtered for 3 hours. This is because the longer the extraction time, the longer the contact time between the solvent and the raw material. So that the process of penetration of the solvent into the raw material cell will be better, and cause more and more compounds to diffuse out of the cell (Wijaya *et al.*, 2019). The extraction results can be seen in **Table 1**.

Table 1. Extraction results and percent yield of the root ethanol extract of the Qust Al Hindi (*Saussurea lappa*) plant

Sample	Amount of Solvent (mL)	Sample Weight (g)	Extract Yield (g)	Extract Rendering (%)
Qust al hindi root	300	50	2,651	5,302

Prior to testing the antioxidant activity of the samples, the maximum wavelength was determined to determine the maximum absorption (**Figure 1**).

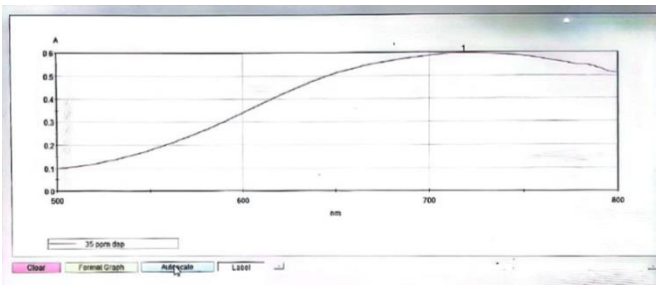

Figure 1. Maximum wavelength measurement results

The result shows the measuring of maximum wavelength of quercetin on a UV-Vis spectrophotometer at around a wavelength of 500-800 nm with a resulting wavelength of 718 nm with an absorbance of 0.596.

The results of the determination of the quercetin standard curve show that the concentration is directly proportional to the absorbance (**Figure 2**). The higher the concentration, the higher the absorbance produced. In picture 2 it shows the results of linear regression of the relationship between concentration and absorbance obtained by a linear equation y = 0.0143x – 0.0191 with a correlation coefficient value (r) = 0.996. The value (r) obtained is close to number 1 indicating that the regression equation is linear, so it can be said that absorbance and concentration have a very strong correlation.

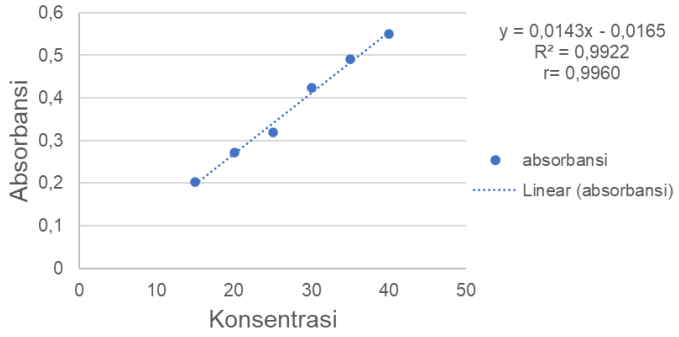

Figure 2. Standard curve of the quercetin standard solution series at a wavelength of 718 nm

The results of the antioxidant activity test of the qust al hindi root ethanol extract using the FRAP method at a wavelength of 718nm, can be seen in **Table 2**. The average antioxidant activity of the qust al hindi ethanol extract made in 3 replications was 10.3671 mgQE/g extract. With a standard deviation of no more than 0.5. In this study the FRAP test on qust al hindi ethanol extract was compared to quercetin, the results obtained were that the antioxidant activity of qust al hindi ethanol extract was still much lower than quercetin. That is, the antioxidant activity of 10.3671 mg of quercetin is equivalent to the antioxidant activity of 1 g of ethanol extract of the root of Qust al Hindi.

Table 2. Antioxidant Activity of Qust Al Hindi Ethanol Extract (*Saussurea lappa*) using the FRAP method

Replication	Sample weight (g)	Sample absorbance (y)	Antioxidant activity (mgQE/g extract)	Average antioxidant activity (mgQE/g extract)	Standard deviation
1	0.0250	0.298	10.9965	10.3671	0.2970
2	0.0250	0.271	10.0524		
3	0.0250	0.271	10.0524		

The FRAP method was chosen based on the ability of antioxidant compounds not only in terms of capturing free radicals but also has one of the advantages, namely being able to determine the total antioxidant content of a material based on the ability of antioxidant compounds to reduce Fe^{3+} to Fe^{2+} (Suhendy *et al.*, 2022). The function of the reagents used in this study is 0.2 M phosphate buffer (pH 6.6) which aims to maintain the pH balance in solution, where it is known

that this complex is stable at acidic pH, so it is used at pH 6.6 (Pratama, M., A. Muflihunna, 2018). In general, the acidic conditions in the FRAP test can reduce the ability to reduce antioxidant compounds due to acid protonation. The ability of quercetin to reduce Fe^{3+} at pH 6.6 is thought to be reduced due to the electrons in ionized oxygen atoms being stabilized better by aromatic systems (Maesaroh *et al*., 2018)The use of pH is intended to facilitate the process of reducing Fe^{3+} to $Fe^{2+.}$ Potassium ferricyanide 1% acts as an oxidizing agent which reacts with the sample which is a reducing agent, so that Fe^{3+} from potassium ferricyanide is reduced to $Fe^{2+.}$ While the addition of 10% trichloroacetate aims to precipitate the potassium complex from potassium ferricyanide, and 0.1% $FeCl_3$ aims to form a green to blue complex (Berlin blue) (Maryam *et al*., 2016).

One feature of the FRAP method is that there is a change in the intensity of the green to sparkling blue color in the sample because the sample provides electron pairs to the FRAP reagent which functions as an oxidizing agent, and the sample acts as a reducing agent to provide electron pairs or donate its hydrogen atoms to free radicals. This color change will give a change in absorbance at its maximum wavelength when measured using a UV-Vis spectrophotometer. So that the value of its antioxidant activity will be known. The greater the intensity of the green color formed in the sample, the higher the absorbance value (Tahir *et al*., 2016). The results of the antioxidant activity of the ethanol extract of the qust al hindi root compared to quercetin showed that its antioxidant activity was very weak compared to quercetin. This is presumably because some of the compounds in the samples that have potential as antioxidants have been damaged by heating. In research conducted by Chang et al., 2012 antioxidant activity test of ethanol extract of qust al hindi extracted at room temperature showed strong antioxidant activity.

4. CONCLUSION

Based on the results of this study, the antioxidant activity of the ethanol extract of the roots of Qust al Hindi (*Saussurea lappa*) has a very weak antioxidant activity when compared to quercetin which already has strong antioxidant activity. That is, the antioxidant activity of 10.3671 mg of quercetin is equivalent to the antioxidant activity of 1 g of ethanol extract of the root of Qust al Hindi. This is suspected. This is presumably because some of the compounds in the samples that have the potential as antioxidants have been damaged by heating. So it is suggested that to improve the results of this study, it is necessary to carry out the extraction process at a lower temperature in order to obtain strong antioxidant activity.

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